

West Nile Virus
Update

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Guidelines for Investigating Suspect West Nile Virus Cases in Equine

Veterinary Services

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Introduction

In the United States, West Nile virus (WNV) has caused disease and deaths in humans, wild birds, zoo birds, and horses. Wild birds are the reservoir for the virus, which is transmitted by mosquitoes. Limiting exposure to mosquitoes and controlling mosquitoes are fundamental in preventing the disease. The purpose of this document is to guide veterinary practitioners and field personnel in investigating and reporting suspect cases of WNV infection in equine.

Equine Precautions

APHIS Veterinary Services (VS) is concerned about horses and other equine because 85 cases of illness in horses have been attributed to WNV infection since the first detection of the virus in the United States in 1999. Thirty-two (38 percent) of those horses died or were euthanatized. Many additional horses that did not develop clinical illness have been found to be infected with WNV based on detection of antibodies to the virus. No transmission of WNV has been documented from horses, either directly or through mosquitoes.

To prevent exposure of equine to WNV, it is necessary to prevent their exposure to mosquitoes. No vaccine for WNV is currently available, although vaccine development is moving forward and a product may soon be approved for use in horses. The most important action to prevent exposure to mosquitoes is source reduction, i.e., the elimination of stagnant water sources where mosquitoes may breed. Insect-proofing stables and other measures that reduce exposure of equine to mosquitoes may be useful in areas where current WNV activity has been documented in mosquitoes, birds, humans, or equine.

Human Precautions

When working with an equine or other mammal showing signs of a central nervous system disorder, always take precautions to avoid exposure to rabies virus. In addition, persons visiting a premises to investigate an unknown disease condition should take measures to prevent exposure to a variety of arthropod-borne zoonotic pathogens. Application of commercially available insect repellents containing DEET to clothing and to exposed parts of the body should be sufficient to protect oneself from mosquitoes carrying WNV.

Equine Surveillance

What should be considered a suspect case of equine WNV infection and how it should be investigated depend on whether or not it occurs in a WNV-affected area. A WNV-affected area is any county

where a WNV infection in an equine has been confirmed in the current calendar year (2001), or any location within 10 miles of a confirmed equine WNV infection. A non-WNV-affected area is any county where WNV infection in equine has not been diagnosed in the current calendar year, or any location more than 10 miles from a positive equine case of WNV infection.

WNV infection in horses may include both central nervous system and peripheral nervous system signs. These signs of disease may be indistinguishable from those produced by other equine encephalitides including rabies, equine herpesvirus-1, equine protozoal myeloencephalitis, and eastern, western, or Venezuelan equine encephalomyelitis. The most common signs of WNV infection in U.S. horses have been ataxia, weakness of limbs, recumbency, muscle fasciculation, and death. Fever has been detected in less than one-quarter of all confirmed cases.

A suspect equine case in a non-WNV-affected area should be investigated as a foreign animal disease (FAD). FAD investigations should be completed in accordance with VS Memorandum 580.4. Specimens should be submitted to the National Veterinary Services Laboratories (NVSL) with an FAD investigation number in order to facilitate tracking and timely reporting of diagnostic results.

Sample Submission

Samples for submission to NVSL should be shipped by Federal Express to:

Dr. Eileen Ostlund
NVSL
1800 Dayton Road
Ames, IA 50010

Contact NVSL (phone: 515-663-7551, fax: 515-663-7348) to provide information on what is being sent. Please report the number and type of samples and relevant epidemiological information, including location of premises (county and closest city), clinical signs observed/reported, date of onset, age of animal, outcome (alive/died/euthanatized), recent travel history, and vaccination status.

Antemortem Sample Collection

Collect one serum sample in a 10 ml red-top tube or clot-separator tube. Send the serum to NVSL. Collection of a whole blood sample (in a 10 ml EDTA purple-top tube) is of less importance but may also be included.

Collection of cerebrospinal fluid (CSF) is of less importance, but if obtained it should be sent to NVSL in a red-top tube labeled with the site of collection (e.g., cervical or lumbosacral).

The NVSL does not perform testing for equine protozoal myeloencephalitis (EPM). If testing for EPM is desired, please retain sufficient quantities of serum or CSF to send for testing at another laboratory of choice.

Postmortem Sample Collection

Use appropriate protective gear when collecting and processing postmortem samples (see below, "Recommendations for Safe Practices for Conducting Necropsies of Suspected WNV Cases").

If a suspect equine is to be euthanatized, collect at least one serum sample in a 10 ml red-top tube or clot-separator tube, prior to euthanasia. Send the serum to NVSL. Collection of a whole blood sample (in a 10 ml EDTA purple-top tube) is of less importance but may also be included.

When a postmortem examination is performed on a suspect equine, the following samples should be collected in priority order listed and sent to NVSL or the State public health laboratory, as indicated:

- Fresh brain tissue (for rabies testing) -- send to State public health laboratory.
- Fresh and fixed brain tissue -- send to NVSL.
- CSF (indicate collection site, e.g., cervical or lumbosacral) -- send to NVSL.
- Fresh and fixed spinal cord segments (cervical, thoracic, and lumbar) -- send to NVSL.

Samples collected from the postmortem examination of a suspect equine and submitted to NVSL for WNV testing will be processed only after the animal has tested negative for rabies according to established protocols in a given State. The foreign animal disease diagnostician should notify NVSL of the rabies test results as soon as they are available.

Recommendations for Safe Practices for Conducting Necropsies of Suspected WNV Cases

WNV is a flavivirus transmitted in nature by mosquitoes. Infection of otherwise healthy people causes a mild febrile illness or no symptoms at all. Mortality has been reported in the elderly; immunocompromised individuals also are at a higher risk.

Although aerosol transmission of WNV is very unlikely, precautions should be taken in laboratory and field settings. The main concern should be to prevent viral contact with open wounds and mucous membranes.

Recommendations for Field Necropsy of WNV Suspect Animals:

1. Keep the use of needles and sharp instruments to a minimum.
2. Do NOT use mechanical saws to obtain spinal cord samples. For proper procedures, see "Collection of Spinal Cord Segments" below.
3. Procedures that create an aerosol should be done in a way to minimize the dispersal of the aerosol particles.

4. Wear Tyvek® disposable coveralls or, at a minimum, a solid-front, water-resistant, long-sleeve gown.

5. Wear three pairs of gloves. The innermost pair should be latex or other disposable gloves. Substantial waterproof gloves (e.g., Playtex® kitchen gloves) should be worn over the innermost pair. The gloves should be long enough for the gown sleeves to be tucked inside the gloves; duct tape may be useful for keeping sleeves inside gloves. The outermost pair of gloves should be metal or Kevlar®, e.g., a Whizard® Hand Guard (steel/Kevlar®) glove from Koch® (1-800-456-5624) or a locally purchased filleting glove. **THIS OUTER PAIR OF GLOVES MUST BE WORN** throughout the necropsy procedure.

6. Wear a face shield or goggles to protect mucous membranes, and wear a disposable "half mask" HEPA respirator (3M® 8293) to avoid aerosol infection.

Collection of Equine Brain Tissue

Diagrams showing the procedure for collecting equine brain tissue are reproduced from Equine Medicine and Surgery, 3rd ed., 1982, edited by Mansmann, McAllister, and Pratt (see the last page of these guidelines). **Always use appropriate protective gear when collecting and processing samples.**

Collection of Spinal Cord Segments

Collect spinal cord in 4-centimeter-long segments from cervical, thoracic, and lumbar sites.

Procedures for Obtaining Cervical Spinal Cord Segments:

1. At the vertebral column where the head has been disarticulated, remove the soft tissue from 4 or 5 cervical vertebrae.

2. Depending on the circumstances, it may be advantageous to disarticulate the cervical vertebral column from the rest of the carcass, allowing the specimen to be placed on an elevated surface for further dissection. Assistance may be needed to hold the specimen on an elevated surface for further dissection. Assistance in holding the specimen steady, in the form of either a person or a vise, will facilitate the remaining steps.

3. Using a manual bone saw, make transverse cuts through the midportion of each of the first four vertebral bodies. This will produce four isolated segments of cervical vertebral column, each containing an intervertebral joint at its center.

4. Observe the isolated vertebral segments from the cut ends, noting the spinal cord held in place by the spinal nerves, which exit the vertebral canal through the intervertebral foramina. Grasp the dura mater

with toothed thumb forceps, apply gentle traction, and snip the spinal nerves with long thin scissors (e.g., Metzenbaums). Perform this procedure at each end of the vertebral segment.

5. For sample submission: divide each cervical spinal cord segment in half; fix one half in formalin and maintain the other half as a fresh sample. Ship the fresh and fixed segments to NVSL.

Procedures for Obtaining Thoracic and Lumbar Spinal Cord Segments:

1. Excise and remove the last two ribs.
2. Remove the soft tissue around the thoracic vertebrae that have had the ribs removed. Also remove the soft tissue from around the adjacent lumbar vertebrae.
3. Basically, repeat the steps used for collecting the cervical spinal cord segments by making transverse cuts through the thoracic vertebrae and continuing down through the exposed lumbar vertebrae.
4. Remove the spinal cord segments from the vertebral segments as described for the cervical cord segments.
5. For sample submission: divide each thoracic and lumbar spinal cord segment in half; fix one half in formalin and maintain the other half as a fresh sample. Ship the fresh and fixed segments to NVSL.

Collection of CSF

A good site to collect CSF is at the atlanto-occipital junction just as one cuts through the ligaments prior to decapitation. Up to 15 ml of CSF can be collected from this site. Collect as much fluid as possible. CSF may also be collected from a sacral tap on postmortem. Identify the CSF as to site of collection and submit to NVSL.

* Mention of a commercial product, trademark, or brand name is for illustrative purposes only and does not constitute endorsement by any individual nor by any agency of the U.S. government.

Procedure for Collecting Equine Brain Tissue

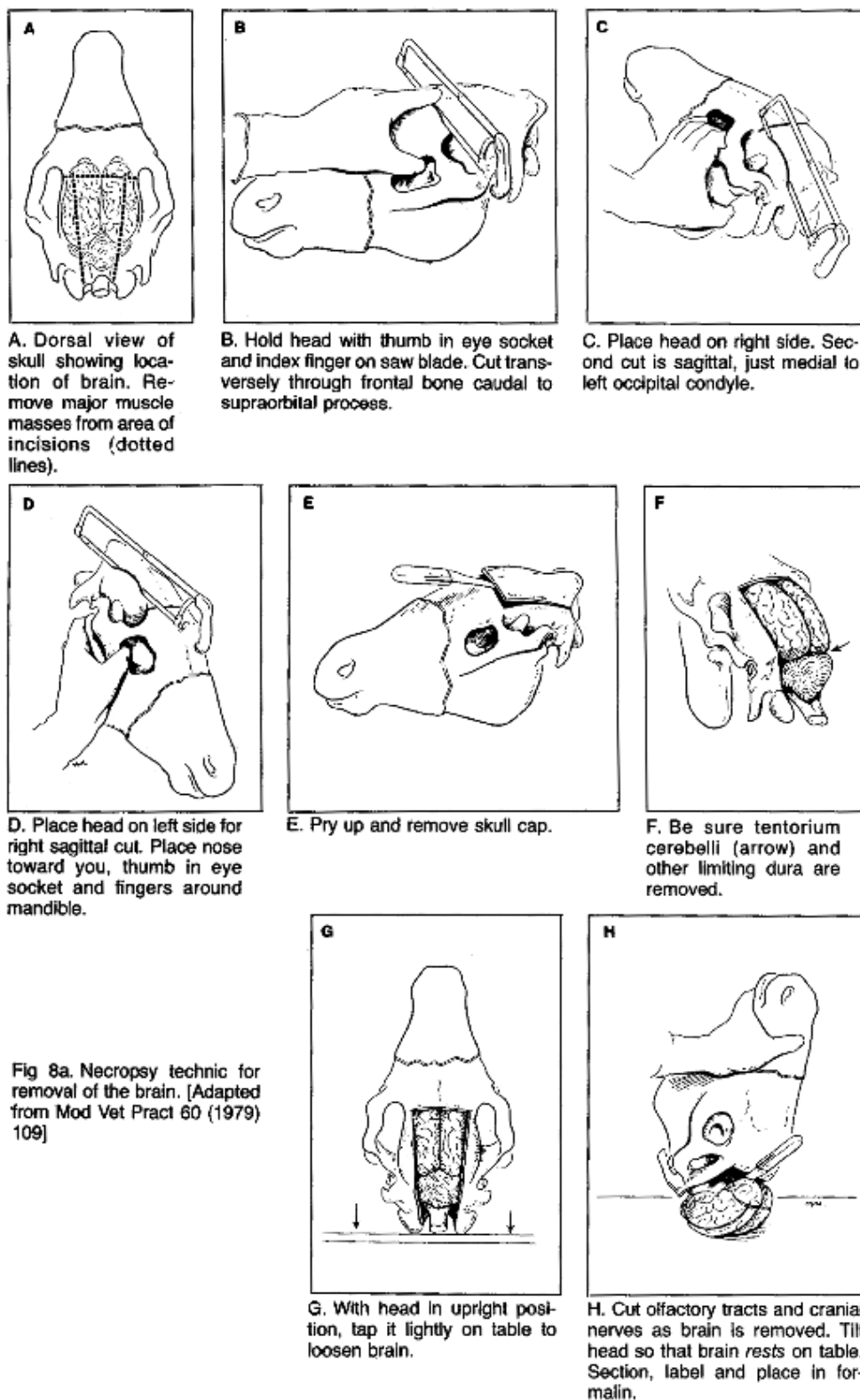


Fig 8a. Necropsy technic for removal of the brain. [Adapted from Mod Vet Pract 60 (1979) 109]

Case Definition for West Nile Virus Infection in Equine

2001

Confirmed Case

Compatible clinical signs^[1] plus one or more of the following:

- isolation of West Nile virus (WNV) from tissues^[2];
- an associated 4-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WNV in appropriately-timed^[3], paired sera;
- detection of both IgM antibody to WNV by IgM-capture ELISA in serum or cerebrospinal fluid (CSF) and an elevated titer (1:10 or greater) to WNV antibody by PRNT in serum;
- detection of both IgM antibody to WNV by IgM-capture ELISA in serum or CSF and a positive polymerase chain reaction (PCR) for WNV genomic sequences in tissues^[2];
- detection of both IgM antibody to WNV by IgM-capture ELISA in serum or CSF and a positive immunohistochemistry (IHC) for WNV antigen in tissue;
- positive IHC for WNV antigen in tissue and a positive PCR for WNV genomic sequences in tissues^[2].

Probable Case ^[4]

Compatible clinical signs^[1] plus one of the following:

- detection of IgM antibody to WNV by IgM-capture ELISA in serum or CSF, but no elevated titer (negative at 1:10) to WNV antibody by PRNT in serum^[5], no positive PCR for WNV genomic sequences tissues^[2], and no positive IHC for WNV antigen in tissue;
- positive PCR for WNV genomic sequences in tissues^[2];
- positive IHC for WNV antigen in tissue.

Notes:

- 1) Clinical signs must include ataxia (including stumbling, staggering, wobbly gait, or incoordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.
- 2) Preferred diagnostic tissues from equine are brain or spinal cord; although tissues may include blood or CSF, the only known reports of WNV isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.
- 3) The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
- 4) An equine classified as a probable case should, if possible, undergo further diagnostic testing to confirm or rule out WNV as the cause of clinical illness.
- 5) A negative PRNT on serum collected 22 days or more after onset of clinical illness will reclassify this equine as a non-case.

Assumptions on which case definitions are based:

- IgM-capture ELISA testing may be slightly nonspecific; cross reactions to closely related flaviviruses (e.g., St. Louis encephalitis virus) may occur.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WNV or a closely related flavivirus has occurred, very likely within the last three months.
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WNV; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.
- Neutralizing antibody detected in serum by PRNT indicates past exposure to WNV; equine exposed to WNV in 1999 or 2000 may test positive for neutralizing antibody by PRNT in 2001.